



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Nuijten et al.

Serial No.: 09/749,025

Filed: December 27, 2000

For: SALMONELLA VACCINE

Confirmation No.: 6121

Examiner: V. Ford

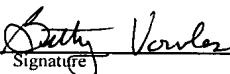
Group Art Unit: 1645

Attorney Docket No.: 2990-5048US

CERTIFICATE OF MAILING

I hereby certify that this correspondence along with any attachments referred to or identified as being attached or enclosed is being deposited with the United States Postal Service as First Class Mail on the date of deposit shown below with sufficient postage and in an envelope addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

April 27, 2007  
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Betty Vowles  
Name (Type/Print)

DECLARATION UNDER 37 C.F.R. § 1.132 OF DR. PIET NUIJTEN

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Dr. Piet Nuijten hereby declares:

1. I am a named inventor on the above-referenced patent application.
2. I am head of the department Bacteriological R&D and Program Manager of humane vaccine projects of Nobilon International B.V. A copy of my curriculum vitae is attached.
3. I understand that in the Office Action mailed October 20, 2006, the Examiner has questioned the fact that the application is enabling for a vaccine composition for the protection against Salmonellosis comprising an immunologically effective amount of any *Salmonella* mutated bacterium wherein the mutated bacterium lack flagellin and wherein the mutated bacterium is attenuated. I also understand that the claims at-issue have been amended and no

longer claim a "vaccine."

4. The as-filed specification is enabling because it includes working examples of non-flagellated mutant *Salmonella* compositions which reduce colonization rates in both chickens and pigs. Specifically, Example 3 of the specification demonstrates that chickens vaccinated with a non-motile mutant of *S. typhimurium* STMP, called *S. typhimurium* STM2000, had reduced colonization of the intestinal tract.

5. Example 4, at pages 22-23 of the specification, shows that the live attenuated flagella-less *S. typhimurium* STM2000 vaccine significantly reduced fecal shedding in pigs after a challenge infection with a wild-type *S. typhimurium* serotype.

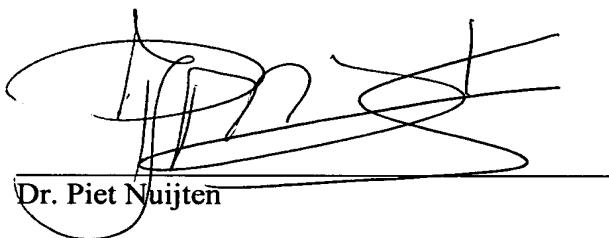
6. The specification also provides detailed instructions for selecting non-motile mutants from serotype *S. typhimurium* SL3261. (Example 1, page 17). In this example, a flagellin protein gene of *S. typhimurium* SL3261 was chemically mutagenized with NTG and non-motile mutants were selected by light microscopy. The selected mutant was named STM2001 and subsequent electrophoretic analysis revealed that the mutant lacked the flagellin protein fragment of 51kDa and pI 4.7, as compared to the non-mutant parent serotype. *Id.*

7. Attached hereto, I present *in vivo* data using four different strains of *Salmonella enterica* bacteria (*S. typhimurium*, *S. enteritidis*, *S. anatum* and *S. hadar*) confirming reduced colonization of wild-type Salmonella in the cloacae of chickens after they were given *Salmonella enterica* fla<sup>-</sup> strains. Therefore, it is submitted that the application provides the skilled person with sufficient guidance to make and use the claimed compositions.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

**Serial No. 09/749,025**

Date: April 23, 2007



A handwritten signature in black ink, appearing to read "Dr. Piet Nijten". The signature is fluid and cursive, with a large, stylized 'D' at the beginning. It is enclosed within a thin-lined oval.

Dr. Piet Nijten

**Personal data.**

Name: Petrus Johannes Maria (Piet) Nuijten  
Address: Radioweg 1  
City: Sambeek  
Zipcode: 5836CD  
Country: The Netherlands  
Nationality: Dutch  
Place of birth: Bergen op Zoom, The Netherlands  
Date of birth: October 5<sup>th</sup> 1959

**Role:**

Head of Department Bacteriological R&D and program manager of human vaccine projects, Nobilon International BV, Boxmeer, The Netherlands.

**Academics:**

1979-1987	Wageningen University, Netherlands: biochemistry, molecular virology, molecular bacteriology. 1985-1986: Master training period in University of Kentucky, Lexington, USA.
1987-1991	Veterinary Faculty, Utrecht University, Netherlands. Ph.D. Project: Molecular Pathogenesis of Campylobacter jejuni. Ph.D. Thesis
1991	
1993-1997	Supervisor Ph.D. Project. Veterinary Faculty, Utrecht University. M. Kolkman. Capsular polysaccharide synthesis in Streptococcus pneumoniae. Thesis Dec 4 <sup>th</sup> 1997.
1994-2000	Supervisor Ph.D. Project.. Institute for Animal Sciences, Lelystad. A. Lammers Pathogenesis of Staphylococcus aureus mastitis. Thesis Oct 5 <sup>th</sup> 2000.
2000-2004	Supervisor Ph.D. Project. Intervet International BV, Boxmeer. D. Schuijffel. A strategic approach for immunity- based selection of cross-protective Ornithobacterium rhinotracheale antigens. Thesis March 17 <sup>th</sup> 2005.

**Professional history:**

1991-1993	Veterinary Faculty, Utrecht University, Netherlands.
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	Post-doc Project: Biosynthesis pathways of capsule polysaccharides in <i>Streptococcus pneumoniae</i> . Medical Faculty, Stanford University, Palo Alto, USA.
1993-1994	Post-doc Project: Identification of toxins in <i>Helicobacter pylori</i> Institute for Animal Sciences, Lelystad, Netherlands.
1994-1996	Post-doc Project: Virulence mechanisms of <i>Staphylococcus aureus</i> in mastitis. Intervet International BV, Molecular Bacteriology, Boxmeer, Netherlands. Animal vaccines. Project leader of many different projects: <i>Salmonella</i> , <i>Haemophilus</i> , <i>Staphylococcus</i> , <i>Streptococcus</i> , <i>Campylobacter</i> , <i>Mycoplasma</i> , Nobilon International BV. Boxmeer, Netherlands.
1996-2004	Human vaccines. Head Bacteriological R&D and Program manager of several vaccine projects.
2004-	

### Publications:

One page.

<input type="checkbox"/> 1: <a href="#">Pot RG, Stoof J, Nuijten PJ, de Haan LA, Loeffen P, Kuipers EJ, van Vliet AH, Kusters JG.</a>	Related Articles, Links
<input checked="" type="checkbox"/> UreA2B2: a second urease system in the gastric pathogen <i>Helicobacter felis</i> . FEMS Immunol Med Microbiol. 2007 Feb 12; [Epub ahead of print] PMID: 17298583 [PubMed - as supplied by publisher]	
<input type="checkbox"/> 2: <a href="#">Schuijffel DF, Van Empel PC, Segers RP, Van Putten JP, Nuijten PJ.</a>	Related Articles, Links
<input checked="" type="checkbox"/> Vaccine potential of recombinant <i>Ornithobacterium rhinotracheale</i> antigens. Vaccine. 2006 Mar 10;24(11):1858-67. Epub 2005 Oct 24. PMID: 16318896 [PubMed - indexed for MEDLINE]	
<input type="checkbox"/> 3: <a href="#">Schuijffel DF, van Empel PC, Pennings AM, van Putten JP, Nuijten PJ.</a>	Related Articles, Links
<input checked="" type="checkbox"/> Successful selection of cross-protective vaccine candidates for <i>Ornithobacterium rhinotracheale</i> infection. Infect Immun. 2005 Oct;73(10):6812-21. PMID: 16177359 [PubMed - indexed for MEDLINE]	
<input type="checkbox"/> 4: <a href="#">Schuijffel DF, van Empel PC, Pennings AM, van Putten JP, Nuijten PJ.</a>	Related Articles, Links
<input checked="" type="checkbox"/> Passive immunization of immune-suppressed animals: chicken antibodies protect against <i>Ornithobacterium rhinotracheale</i> infection. Vaccine. 2005 May 16;23(26):3404-11. PMID: 15837364 [PubMed - indexed for MEDLINE]	
<input type="checkbox"/> 5: <a href="#">Moller AK, Leatham MP, Conway T, Nuijten PJ, de Haan LA, Krogfelt KA, Cohen PS.</a>	Related Articles, Links

 5: An Escherichia coli MG1655 lipopolysaccharide deep-rough core mutant grows and survives in mouse cecal mucus but fails to colonize the mouse large intestine.  
Infect Immun. 2003 Apr;71(4):2142-52.  
PMID: 12654836 [PubMed - indexed for MEDLINE]

6: [Nuijten PJ, van den Berg AJ, Formentini I, van der Zeijst BA, Jacobs AA.](#) Related Articles, Links

 7: DNA rearrangements in the flagellin locus of an flaA mutant of *Campylobacter jejuni* during colonization of chicken ceca.  
Infect Immun. 2000 Dec;68(12):7137-40.  
PMID: 11083841 [PubMed - indexed for MEDLINE]

7: [Jacobs AA, Goovaerts D, Nuijten PJ, Theelen RP, Hartford OM, Foster TJ.](#) Related Articles, Links

 8: Investigations towards an efficacious and safe strangles vaccine: submucosal vaccination with a live attenuated *Streptococcus equi*.  
Vet Rec. 2000 Nov 11;147(20):563-7.  
PMID: 11104039 [PubMed - indexed for MEDLINE]

8: [Allen JH, Utley M, van Den Bosch H, Nuijten P, Witvliet M, McCormick BA, Krogfelt KA, Licht TR, Brown D, Mauel M, Leatham MP, Laux DC, Cohen PS.](#) Related Articles, Links

 9: A functional cra gene is required for *Salmonella enterica* serovar typhimurium virulence in BALB/c mice.  
Infect Immun. 2000 Jun;68(6):3772-5.  
PMID: 10816546 [PubMed - indexed for MEDLINE]

9: [Schijns VE, Weining KC, Nuijten P, Rijke EO, Staeheli P.](#) Related Articles, Links

 10: Immunoadjuvant activities of *E. coli*- and plasmid-expressed recombinant chicken IFN-alpha/beta, IFN-gamma and IL-1beta in 1-day- and 3-week-old chickens.  
Vaccine. 2000 Apr 14;18(20):2147-54.  
PMID: 10715530 [PubMed - indexed for MEDLINE]

10: [Lammers A, Kruijt E, van de Kuijt C, Nuijten PJ, Smith HE.](#) Related Articles, Links

 11: Identification of *Staphylococcus aureus* genes expressed during growth in milk: a useful model for selection of genes important in bovine mastitis?  
Microbiology. 2000 Apr;146 ( Pt 4):981-7.  
PMID: 10784056 [PubMed - indexed for MEDLINE]

11: [Lammers A, Nuijten PJ, Smith HE.](#) Related Articles, Links

 12: The fibronectin binding proteins of *Staphylococcus aureus* are required for adhesion to and invasion of bovine mammary gland cells.  
FEMS Microbiol Lett. 1999 Nov 1;180(1):103-9.  
PMID: 10547450 [PubMed - indexed for MEDLINE]

12: [Lammers A, Nuijten PJ, Kruijt E, Stockhove-Zurwieden N, Vecht U, Smith HE, van Zijderveld FG.](#) Related Articles, Links

 13: Cell tropism of *Staphylococcus aureus* in bovine mammary gland cell cultures.  
Vet Microbiol. 1999 Jun 15;67(2):77-89.  
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13: [Kolkman MA, van der Zeijst BA, Nuijten PJ.](#) Related Articles, Links

 14: Diversity of capsular polysaccharide synthesis gene clusters in *Streptococcus pneumoniae*.

J Biochem (Tokyo). 1998 May;123(5):937-45.  
PMID: 9562629 [PubMed - indexed for MEDLINE]

14: [Kolkman MA, Wakarchuk W, Nuijten PJ, van der Zeijst BA.](#) Related Articles, Links  
 Capsular polysaccharide synthesis in *Streptococcus pneumoniae* serotype 14: molecular analysis of the complete cps locus and identification of genes encoding glycosyltransferases required for the biosynthesis of the tetrasaccharide subunit. *Mol Microbiol*. 1997 Oct;26(1):197-208.  
PMID: 9383201 [PubMed - indexed for MEDLINE]

15: [Kolkman MA, van der Zeijst BA, Nuijten PJ.](#) Related Articles, Links  
 Functional analysis of glycosyltransferases encoded by the capsular polysaccharide biosynthesis locus of *Streptococcus pneumoniae* serotype 14. *J Biol Chem*. 1997 Aug 1;272(31):19502-8.  
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16: [Kolkman MA, Morrison DA, Van Der Zeijst BA, Nuijten PJ.](#) Related Articles, Links  
 The capsule polysaccharide synthesis locus of *streptococcus pneumoniae* serotype 14: Identification of the glycosyl transferase gene cps14E. *J Bacteriol*. 1996 Jul;178(13):3736-41.  
PMID: 8682774 [PubMed - indexed for MEDLINE]

17: [Nuijten PJ, Marquez-Magana L, van der Zeijst BA.](#) Related Articles, Links  
 Analysis of flagellin gene expression in flagellar phase variants of *Campylobacter jejuni* 81116. *Antonie Van Leeuwenhoek*. 1995;67(4):377-83.  
PMID: 7574555 [PubMed - indexed for MEDLINE]

18: [Wassenaar TM, Bleumink-Pluym NM, Newell DG, Nuijten PJ, van der Zeijst BA.](#) Related Articles, Links  
 Differential flagellin expression in a flaA flaB+ mutant of *Campylobacter jejuni*. *Infect Immun*. 1994 Sep;62(9):3901-6.  
PMID: 8063406 [PubMed - indexed for MEDLINE]

19: [Cawthraw S, Ayling R, Nuijten P, Wassenaar T, Newell DG.](#) Related Articles, Links  
 Isotype, specificity, and kinetics of systemic and mucosal antibodies to *Campylobacter jejuni* antigens, including flagellin, during experimental oral infections of chickens. *Avian Dis*. 1994 Apr-Jun;38(2):341-9.  
PMID: 7526839 [PubMed - indexed for MEDLINE]

20: [Nuijten PJ, van der Zeijst BA, Newell DG.](#) Related Articles, Links  
 Localization of immunogenic regions on the flagellin proteins of *Campylobacter jejuni* 81116. *Infect Immun*. 1991 Mar;59(3):1100-5.  
PMID: 1705240 [PubMed - indexed for MEDLINE]

21: [Nuijten PJ, Bartels C, Bleumink-Pluym NM, Gaastra W, van der Zeijst BA.](#) Related Articles, Links  
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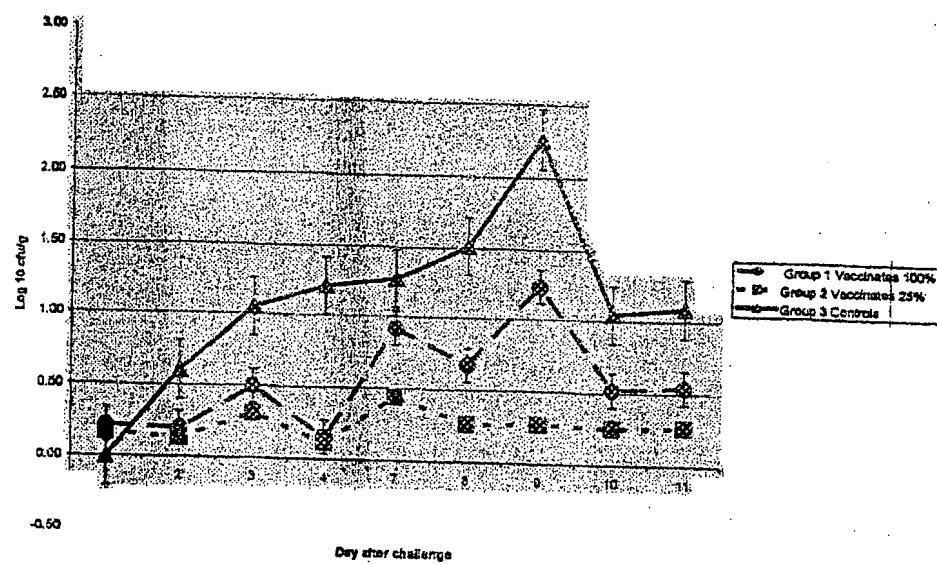
22: [Nuijten PJ, van Asten FJ, Gaastra W, van der Zeijst BA.](#) Related Articles, Links  
 Structural and functional analysis of two *Campylobacter jejuni* flagellin genes.  
J Biol Chem. 1990 Oct 15;265(29):17798-804.  
PMID: 2211662 [PubMed - indexed for MEDLINE]

23: [Nuijten PJ, Bleumink-Pluym NM, Gaastra W, van der Zeijst BA.](#) Related Articles, Links  
 Flagellin expression in *Campylobacter jejuni* is regulated at the transcriptional level.  
Infect Immun. 1989 Apr;57(4):1084-8.  
PMID: 2466792 [PubMed - indexed for MEDLINE]

**SUMMARY**

Group 1 of 30 SPF layers were vaccinated i/m at 4 weeks of age and again at 8 weeks of age with an experimental vaccine comprising  $2 \times 10^9$  killed cells of each of F11C mutants of S.Typhimurium, S.Enteritidis, S. Anatum and S. Hadar in 25% alhydrogel with thiomersal as preservative. Group 3 (30 birds) was not vaccinated and Group 2 (30 birds) was given a vaccine which contained 25% of the amounts of each antigen. At 12 weeks of age all birds were challenged orally with  $10^7$  cfu of a different strain of wild type S. Anatum and this inoculation was repeated on the 2 subsequent days (i.e. 3 lots of  $10^7$  cfu over 3 days). Cloacal samples were tested for the presence of S. Anatum for 11 days. The extent of S. Anatum colonisation was expressed as the group mean number of S. Anatum cfu/g of cloacal sample. S. Anatum colonisation in group 1 (figure) was significantly less ( $P < 0.05$ ) than seen in group 3 (figure) or group 2. S. Anatum colonisation in group 2 was also significantly less than in group 3 (figure).

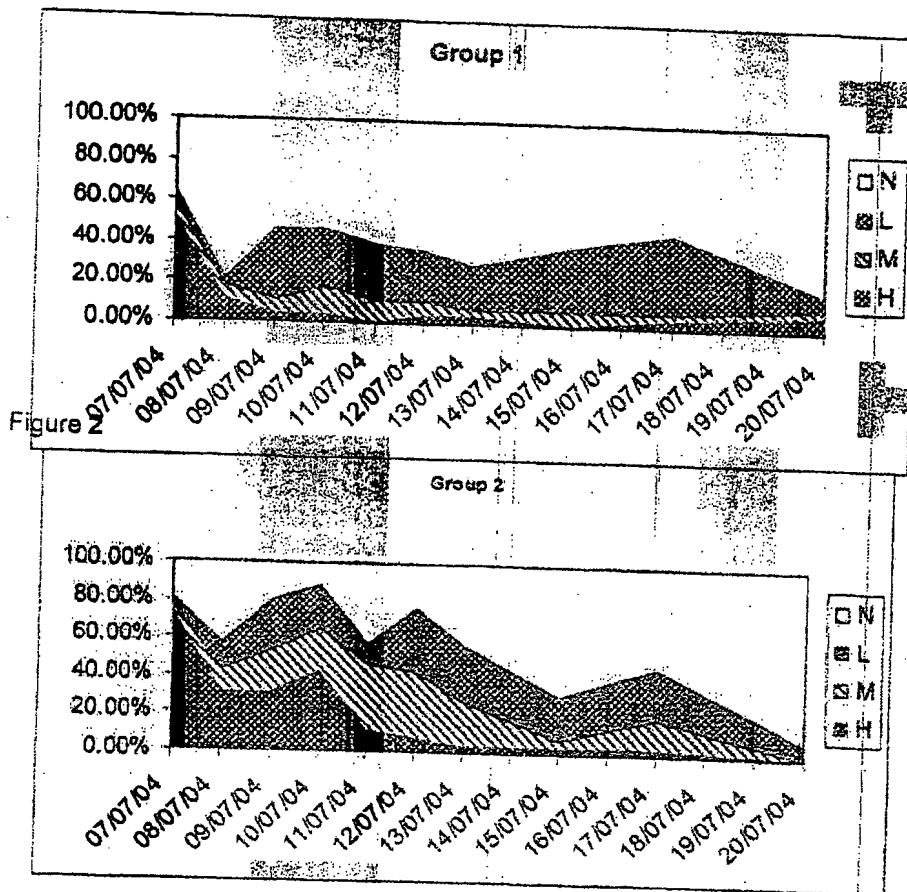
Figure 1. Numbers of S. Anatum recovered from cloacal swabs.



**SUMMARY**

Group 1 (28 SPF layers) were vaccinated i/m at 4 weeks of age and again at 8 weeks of age with an experimental vaccine comprising  $2 \times 10^9$  killed cells of each of fliC mutants of S.Typhimurium, S.Enteritidis, S. Anatum and S. Hadar in 25% alhydrogel with thiomersal as preservative. Group 2 (28 birds) was not vaccinated and Group 3 (28 birds) was given a vaccine which contained 25% of the amounts of each antigen. At 12 weeks of age all birds were challenged orally with  $10^9$  cfu of a different strain of wild type S.Enteritidis. Cloacal samples were tested for the presence of S.Enteritidis for 14 days. The amount of contamination recovered on culture medium was characterised as negative (N), low (L), medium (M) or high (H). The extent of S.Enteritidis colonisation was expressed as the percentage of birds with different levels of contamination. S.Enteritidis colonisation in group 1 (figure 1) was significantly less ( $P < 0.05$ ) than seen in group 2 (figure 2) or group 3 (not shown: group 3 not significantly different from group 2).

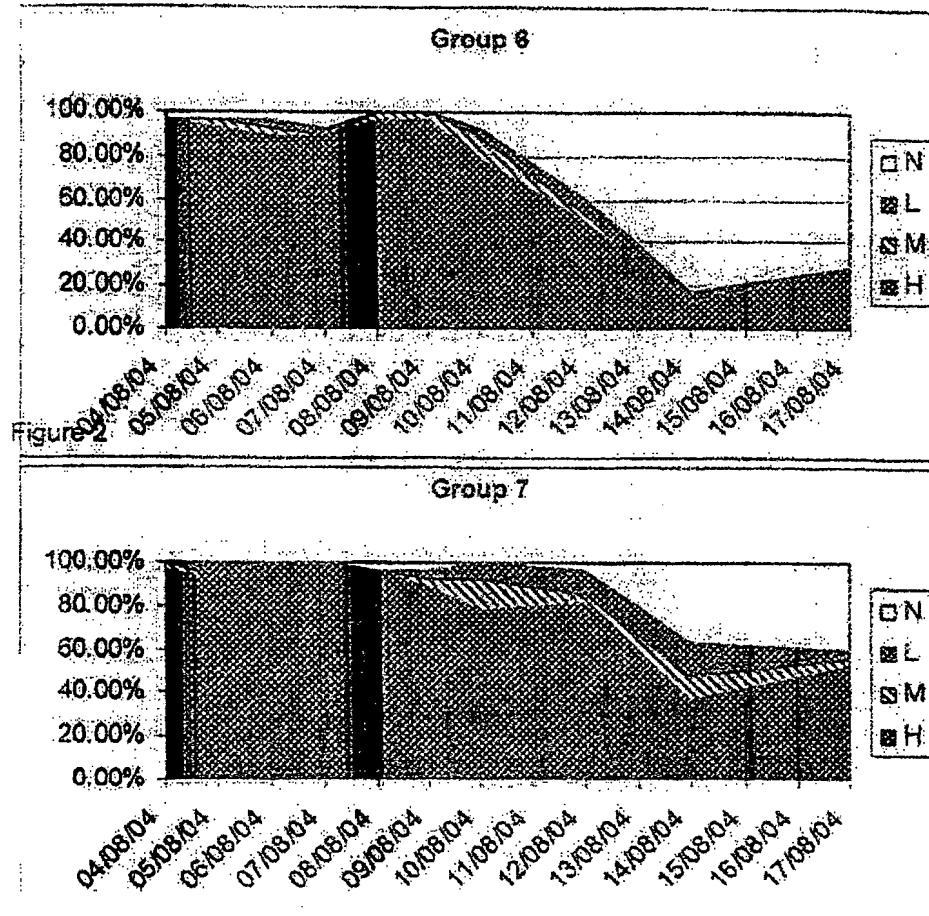
Figure 1



**SUMMARY**

Group 6 (28 SPF layers) were vaccinated i/m at 4 weeks of age and again at 8 weeks of age with an experimental vaccine comprising  $2 \times 10^9$  killed cells of each of fliC mutants of S.Typhimurium, S.Enteritidis, S. Anatum and S. Hadar in 25% alhydrogel with thiomersal as preservative. Group 7 (28 birds) was not vaccinated and Group 8 (28 birds) was given a vaccine which contained 25% of the amounts of each antigen. At 12 weeks of age all birds were challenged orally with  $10^9$  cfu of a different strain of wild type S. Hadar. Cloacal samples were tested for the presence of S. Hadar for 14 days. The amount of contamination recovered on culture medium was characterised as negative (N), low (L), medium (M) or high (H). The extent of S. Hadar colonisation was expressed as the percentage of birds with different levels of contamination. S. Hadar colonisation in group 6 (figure 1) was significantly less ( $P < 0.05$ ) than seen in group 7 (figure 2) or group 8 (not shown: group 8 not significantly different from group 7).

Figure



**SUMMARY**

Group 11 (14 SPF layers) were vaccinated i/m at 4 weeks of age and again at 8 weeks of age with an experimental vaccine comprising  $2 \times 10^9$  killed cells of each of fliC mutants S.Typhimurium, S.Enteritidis, S. Anatum and S. Hadar in 25% alhydrogel with thiomersal as preservative. Group 12 (14 birds) was not vaccinated. At 12 weeks of age all birds were challenged orally with  $10^9$  cfu of a different strain of wild type S.Typhimurium and this inoculation was repeated on the 2 subsequent days (i.e. 3 lots of  $10^9$  cfu over 3 days). Cloacal samples were tested for the presence of S.Typhimurium for 11 days. The amount of contamination recovered on culture medium was characterised as negative (N), low (L), medium (M) or high (H). The extent of S.Typhimurium colonisation was expressed as the percentage of birds with different levels of contamination. S.Typhimurium colonisation in group 11 (figure 1) was significantly less ( $P < 0.05$ ) than seen in group 12 (figure 2).

Figure 1

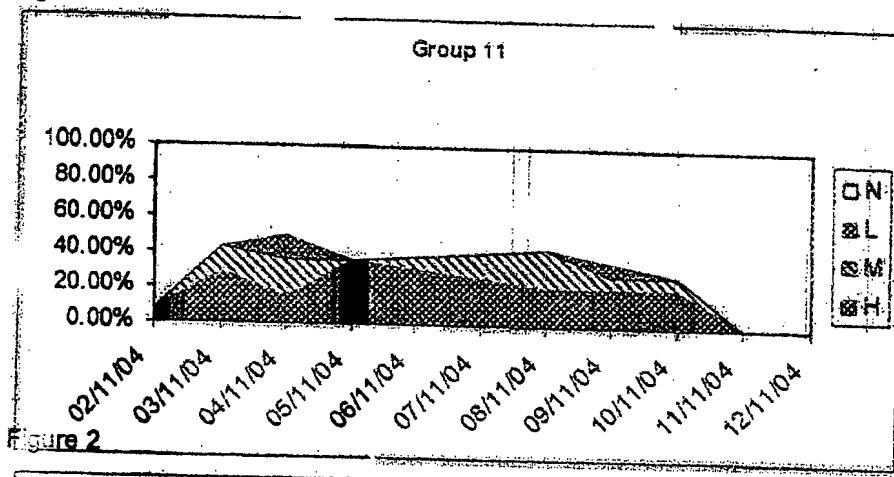


Figure 2

